Secrets of succulence

Jamie Males*

Department of Plant Sciences, University of Cambridge

Downing Street, Cambridge, CB2 3EA, UK

*Correspondence:

jom23@cam.ac.uk

+44(0)7792992635

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Abstract

Succulent plants are iconic components of the florae of many terrestrial ecosystems, but despite having prompted fascination and investigation for centuries, they still harbour many secrets in terms of physiological function and evolution. Tackling these mysteries is important, as this will not only provide insights into the dynamics and details of the convergent evolution of a major adaptive syndrome, but also inform efforts to conserve endangered biodiversity and utilise the unique physiological characteristics of succulents for biofuel and biomass production. Here I review advances in the phylogeny and organismal biology of succulent plants, and discuss how insights from recent work in the wider fields of plant hydraulics and photosynthetic physiology may relate to succulents. The potential for the exploration of mechanistic relationships between anatomical structure and physiological function to improve our understanding of the constraints that have shaped the evolution of succulence is highlighted. Finally, attention is drawn to how new methodologies and technologies provide exciting opportunities to address the wide range of outstanding questions in succulent plant biology.

Introduction

Succulent plants have been the subject of fascination for centuries, but their relevance as masters of water management has perhaps never been greater than now, ~~as~~ with accelerating global change and pressure on natural and agricultural systems urgently demandings ~~urgent~~ insights into the mechanisms of drought-resistance. Understanding the full story of succulent plant biology requires answers to a series of ~~superficially~~ seemingly straightforward, but actually rather challenging, questions. What exactly is succulence? Which plants have evolved succulence, and under what conditions? What selective advantages can succulence confer? What can succulence do for us? In this review, I discuss recent advances towards answering these overarching questions, with a particular emphasis on water relations, and identify a path to take us forwards in the twofold quest both to understand succulent plants and to utilise that understanding in applied contexts. Although some aspects of the distinctive biology of succulent halophytes are briefly discussed, the focus is on classical drought-avoidance succulents (*sensu* Ogburn and Edwards, 2010).

What's in a name? Measuring succulence in its many forms

Succulence is a phenomenon that has long eluded a decisive consensus definition. Traditionally, succulent plants have been treated as a distinct functional group within the plant kingdom. The boundaries defining membership of that group have fluctuated, and quite different terms have been used to define them. Few but the most practical of taxonomists would use the definition of the 18th-century botanist Richard Bradley, who identified succulents as those species which are 'not capable of an Hortus-siccus' (i.e. could not be prepared as herbarium specimens because of their juiciness; Bradley, 1716-1727). The morphological *Gestalt* of succulent plants, as described by Ogburn and Edwards (2010), remains a useful concept because of its familiarity, and the binary discrimination between succulents and non-succulents is often adequate in simple functional type classification schemes. However, it is problematic ~~for~~ in terms of the identification of thresholds. What particular combination of trait values are sufficient to make a plant 'succulent'? Do different succulent plants even conform to a single set of criteria? As will be discussed, superficially equivalent succulent morphologies may be underpinned by strongly contrasting internal

anatomy. Indeed, while succulence is manifested fundamentally at the cellular level, this need not translate to morphological succulence. Ogburn and Edwards (2010) give the example of the bromeliad *Tillandsia usneoides* (L.) L. (Spanish moss), which displays strongly succulent cells, with important consequences for the species' physiological ecology (Kluge et al., 1973), even though the leaves ~~appear~~ (and the whole plant) are highly morphologically reduced.

The enigmatic nature of succulence is perhaps to be expected of any syndrome emerging from variation in quantitative traits (Ogburn and Edwards, 2010). Eggli and Nyffeler (2009) have provided one of the most complete definitions of succulence as the 'storage of utilizable water in living tissues in one or several plant parts in such a way as to allow the plant to be temporarily independent from external water supply but to retain at least some physiological activity'. According to this definition, succulents must be able to use some of the water they have stored through the regulation of processes in living cells. High apoplastic water content is therefore not sufficient qualification. Nor do succulents enter a state of metabolic inactivity during periods of reduced water availability, as is the case with resurrection plants (Farrant and Moore, 2011). These characteristics sum to make succulent plants classical examples of drought-avoiders (Eggli and Nyffeler, 2009; Ogburn and Edwards, 2010).

Some of the many proposed metrics for succulence have been discussed by Von Willert et al. (1990) and Ogburn and Edwards (2010, 2012~~3~~). While some are based simply on water content, others take into account tissue structure and chemical composition or other anatomical parameters. The easily-quantifiable saturated water content (SWC; Ogburn and Edwards, 2012), which is the ratio of water mass at full hydration to dry tissue mass, is gaining traction in comparative studies. As with all such indices, of prime importance is the principle of comparability. Is what makes one species succulent the same as what makes another species succulent? To answer this, one must consider some of the structural diversity that exists among succulent plants.

Anatomical and morphological diversity

Succulence can occur in any vegetative organ. Although leaf- and stem-succulence are most familiar, water storage may also occur in roots, the bulbs or tubers of geophytes, orchid pseudobulbs, and the parenchymatous rays of pachycaul trees (Eggli and Nyffeler, 2009, 2010). Although most physiological research has focussed on stem- and leaf-succulence, Hearn et al. (2013) have shown a high degree of phylogenetic coordination between origins of aboveground and belowground succulence across the eudicots. This suggests that evolutionary transitions in the organ-specificity of succulence can occur quite readily, which in turn points to a common developmental basis of succulence in different plant parts. Within specific organs, succulence can arise from different tissues. For instance, in succulent *Peperomia* Ruiz & Pav. (Piperaceae) it is primarily the epidermal layers that are involved in water storage (Kaul, 1977), whereas in succulent bromeliads it is the hypodermal layer that has been co-opted for this function (Tomlinson, 1969).

Among species with photosynthetic succulent stems and leaves, two main types of anatomical arrangement prevail. Ihlenfeldt (1985) termed these *Allzellsukkulenz* ('all-cell succulence') and *Speichersukkulenz* ('storage succulence'). The former term describes the situation where water is stored in enlarged photosynthetic cells, whereas the latter describes a division of labour between photosynthetic tissues and specialised water storage tissues (hydrenchyma). Fig. 1 illustrates some arrangements of chlorenchyma and hydrenchyma that occur in different leaf-succulent lineages. In Fig. 1a, a typical all-cell succulent leaf structure is shown, which involves a comparatively homogenous structure throughout the leaf. The arrangement in Fig. 1b, with a central core of hydrenchyma transitioning either gradually or abruptly into a peripheral rind of chlorenchyma, is typical of many monocot leaf-succulents in the Asparagales (e.g. *Aloë* spp. and *Agave* L. spp.). In some succulent groups (e.g. Piperaceae), the reverse arrangement often occurs, with a peripheral layer of hydrenchyma encircling a central core of chlorenchyma. Meanwhile all-cell succulence in the chlorenchyma combined with a well-developed adaxial layer of hydrenchyma is characteristic of many bromeliad species, where the transition between the chlorenchyma and hydrenchyma can be either abrupt (as in Fig. 1c) or more gradual. While it seems likely that this extensive structural variation could account for ecophysiological divergences among leaf-succulents, attempts to definitively draw together interacting structure-function relationships in three-dimensional tissues are only now becoming

possible through the emergence of new visualisation and modelling methodologies (Brodersen and Roddy, 2016; Ho et al., 2016).

[FIGURE 1]

However, Ihlenfeldt (1985) made several suggestions as to the functional significance of the distinction between all-cell succulence and storage succulence in ~~, discussed here in the context of~~ leaves. First, all-cell succulence should be self-limiting with respect to organ size. A larger leaf will hold more water and have a lower surface area-to-volume ratio (SA:V), reducing the ratio of transpiration to hydraulic capacitance. However, thicker tissues impose stronger constraints on the diffusion of CO_2 from stomata to chloroplasts, such that assimilation in the centre of the leaf may be inefficient (Maxwell et al., 1997). Perhaps for this reason, all-cell succulence generally occurs in species with small, non-spheroid leaves with a higher SA:V. This has important implications for leaf economics, thermal physiology and light relations. Ihlenfeldt (1985) also remarked that all-cell succulents can only lose a limited amount of water content before experiencing physiological dysfunction, since water loss must necessarily occur from photosynthetically-active cells.

Meanwhile, Ripley et al. (2013) have demonstrated that storage-type anatomy can be associated with relatively high chlorenchyma CO_2 conductance (g_m). The segregation of photosynthetic and water storage functions thus allows g_m and photosynthetic capacity to be decoupled from total leaf water content. However, despite this advantage, storage succulence requires investment in mechanical adaptations at a considerable carbon cost (Von Willert et al., 1990), often including a rigid epidermal-hypodermal complex, which Ihlenfeldt (1985) described as a supportive 'exoskeleton'. Although comparative physiological data are limited, all-cell succulents are generally thought to occupy a position closer to the ecologically opportunistic 'live fast, die young' end of the leaf economics spectrum when compared with the more conservative and less flexible storage succulents (Ihlenfeldt, 1985; Von Willert et al., 1990). Many succulents display a combination of all-cell and storage succulence, including members of the Bromelioideae (Bromeliaceae; Tomlinson, 1969).

Gross morphology, particularly SA:V, is an important determinant of functional succulence. Working with columnar cacti, Williams et al. (2014) elucidated the quantitative links

between species-specific stem SA:V, which is constrained by a trade-off between area-based water loss and water storage capacity, and bioclimatic relations. Insights from stable isotope analyses have recently added a third dimension to the picture for cacti: photosynthetic capacity, which is constrained by diffusive and optical trade-offs to evolve in coordination with morphology and climate envelope (Hultine et al., 2016).

Leaf temperature is one of the many ecophysiological variables with which succulence interacts through morphology (Nobel, 1988; Von Willert et al., 1992). Both modelling (e.g. Leigh et al., 2012) and empirical work (Larcher et al., 2010; Monteiro et al., 2016) have highlighted the importance of leaf thickness and density for maintaining sub-critical leaf temperature under strong environmental forcing. Additionally, temperature gradients within leaves have recently been implicated in the magnitude of vapour-phase fluxes of water from evaporative sites to the stomatal pore (Rockwell et al., 2014; Buckley, 2015; Buckley et al., 2017). These gradients are likely to be particularly steep in succulent leaves with high thermal capacity. It is possible that some evolutionary origins of succulence may have been promoted by~~related in part to~~ a selective advantage associated with the suppression of the potential for large vapour-phase fluxes.

Phylogenetic and biogeographic diversity

The numerous origins of succulence scattered across the land plant phylogeny are frequently cited as a classic example of morphological (if not anatomical and functional) convergence. While succulence is by no means limited to the angiosperms (it occurs, for instance, in *Pyrrosia* Mirb. ferns and the gymnosperm *Welwitschia* Hook.f.), the majority of succulents are flowering plants. Succulents are widely distributed across the angiosperm phylogeny, offering extensive evolutionary replication for investigators (Ogburn and Edwards, 2010). Recently, advances have been made in clarifying phylogenetic relationships within several major succulent lineages, including *Aloë* L. (Asphodelaceae; Grace et al., 2015), *Euphorbia* L. (Euphorbiaceae; Horn et al., 2012; Peirson et al., 2013; Evans et al., 2014; Horn et al., 2014), *Opuntia* Mill. (Cactaceae; Majure et al., 2012), and Ruschieae (Aizoaceae; Klak et al., 2013). The Portullugo clade (Caryophyllales) developed as a model system by Edwards and colleagues has proved particularly fruitful for testing evolutionary

hypotheses (Nyffeler et al., 2008; Ogburn and Edwards, 2009, 2013, 2015). However, there is still tremendous scope for integrated progress in the phylogenetics, morphoanatomy and physiology of such critical taxa as the Crassulaceae, Orchidaceae, Asphodelaceae, Asteraceae, Aizoaceae, Apocynaceae and Bromeliaceae. Improved characterisation of the evolutionary trajectories leading to succulence in different lineages would help us to understand the extent of parallelism in independent origins.

[FIGURE 2]

Succulents occur in almost all parts of the world, but centres of diversity are readily identifiable (Fig. 2). The deserts and semi-deserts of southwest North America are rich in iconic stem- succulent cacti and leaf- and stem-succulent agaves and Crassulaceae. The forests of the northern Andes host the greatest concentration of succulent epiphytic bromeliads and orchids, although these are widespread throughout the Neotropics and (in the case of the orchids) other tropical regions. Further south in the Andean cordillera is another succulent hotspot reaching from Peru into Bolivia, where cacti and terrestrial bromeliads are particularly profuse. The florae of the Caatinga and Campo Rupestre regions of Brazil include numerous endemic stem-succulent cacti and euphorbs. The highest succulent diversity occurs in southern Africa's Succulent Karoo, including abundant Aizoaceae, Crassulaceae, caudiciforms and geophytes. In Madagascar caudiciforms are joined by euphorbs and endemic Didiereaceae. Along the North African littoral and on the Macaronesian islands are further radiations of Crassulaceae and Euphorbiaceae, and in the Irano-Turanian floristic region the succulent halophytes of the Chenopodioideae and Zygophyllaceae reach their highest diversity. Other regional florae with notable but less diverse succulent elements include those of Australia and various alpine regions. With the exception of the special case of the northern Andean forests with its diverse epiphyte flora, these hotspots show varying degrees of aridity and seasonality, which are two of the environmental pressures classically associated with succulent growth-forms (Von Willert et al., 1992; Ogburn and Edwards, 2010).

There is great disparity in the species richness of succulent clades. The lone succulent grass species, *Dregeochloa pumila* (Nees) Conert, might be regarded as an evolutionary 'dead-end' when contrasted with the extensive radiations of other succulent monocot groups like *Agave* and *Aloë*, which together comprise over 700 species. The most dramatic succulent

radiations have arisen from what Donoghue and Sanderson (2015) refer to as the 'confluence' (i.e. co-occurrence) of a 'synnovation' and ecological opportunity. 'Synnovation' denotes an ensemble of adaptive innovations that synergistically displace or broaden a population's ecological amplitude. Meanwhile, the ecological opportunity is provided by the favourable alignment of environmental factors opening up highly unsaturated niche space to invasion. Recent research has unearthed several examples of this scenario, including *Agave*, the Aizoaceae, terrestrial Bromeliaceae, Cactaceae and Euphorbiaceae, all of which independently evolved a synnovation complex involving succulence and Crassulacean acid metabolism (CAM). In each case, this synnovation complex was closely linked to exploitation of the large geographical regions of semi-arid climate that arose during the global climatic changes between the late Oligocene and late Miocene (Horn et al., 2014; Good-Avila et al., 2006; Arakaki et al., 2011; Givnish et al., 2014; Hernández-Hernández et al., 2014; Valente et al., 2014). Parallel and contemporaneous selective pressures therefore appear to have been important in shaping the present-day diversity of succulent plants. However, other innovations, including new habits and growth-forms (Givnish et al., 2014; Hernández-Hernández et al., 2014; Givnish et al., 2015; Freudenstein and Chase, 2015) and environmental and biotic factors, including forest dynamics (Xiang et al., 2016) and pollinator coevolution (Hernández-Hernández et al., 2014; Givnish et al., 2015; Freudenstein and Chase, 2015), have sometimes been critical.

Succulence and plant economic relationships

Succulence does not represent a single peak on a simple adaptive landscape, because it assumes many primary and secondary functions, ranging from short- to long-term water-storage, and from salt accumulation to thermal insulation. Succulence is compatible with occupation of a range of positions along the plant economic spectrum (Reich, 2014), with many storage succulents being slow-growing stress-tolerators, and all-cell succulents being more resource-acquisitive. The diversity of economic strategies displayed by succulents can be expanded even further when drought-deciduous leaf-succulents and deciduous leaf-succulent geophytes are considered (e.g. Von Willert et al., 1990; Donatz and Eller, 1993; Wiegand et al., 2000). Moreover, the transformative effect of succulence on structure and function is reflected in the way it tends to distort plant economic relationships (Vendramini

et al., 2002). For example, the classical correlation between photosynthetic capacity and leaf mass per unit area (LMA; Wright et al., 2004) is notably weaker in leaf-succulents than in other plant groups (Ripley et al., 2013; Grubb et al., 2015). This is because investment in differentiated hydrenchyma introduces an additional source of variation in LMA, but may have comparatively little effect on the photosynthetic capacity of the chlorenchyma. Thus, by rewiring trait networks, origins of succulence can reshape the constraints on functional trait evolution. This important effect could allow new trait combinations to arise and thereby act as a pump for the evolution of ecophysiological diversity.

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Among the angiosperms, leaf-succulence is perhaps the most phylogenetically widespread form of succulence at the familial level, with instances of stem- and root-succulence, pachycauly, and succulent tubers or bulbs scattered across the major clades (Nyffeler and Eggli, 2010). However, there is extensive structural variation both between and within families expressing each of these types of succulence. As an example, Fig. 2 illustrates some arrangements of chlorenchyma and hydrenchyma that occur in different leaf storage-succulent lineages. The arrangement in Fig. 2a, with a central, sharply-defined core of hydrenchyma, is typical of *Aloë* spp., whereas a more gradual transition between tissue types is common in *Agave* spp. (Fig. 2b). A well-developed adaxial layer of hydrenchyma is characteristic of many bromeliad species, where its transition into the chlorenchyma can be either abrupt (Fig. 2c) or gradual (Fig. 2d). In some Piperaceae there is a peripheral layer of hydrenchyma encircling a central core of chlorenchyma (Fig. 2e). While it is intuitive that this extensive structural variation could account for ecophysiological divergences among leaf-succulents, attempts to definitively draw together interacting structure-function

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Selection for succulence

High degrees of succulence have traditionally been associated with regions of low, seasonal rainfall, and many succulent plants conform to the stereotype of a large, slow-growing perennial in a semi-arid habitat, including most succulent Cactaceae and Euphorbiaceae. However, Ogburn and Edwards (2015) recently demonstrated that in the Montiaceae there is no relationship between succulence (quantified as SWC) and precipitation seasonality, although SWC did correlate negatively with mean annual precipitation. This highlights the need to move on from limiting generalisations. The achievement of a comprehensive understanding of the relationship between succulence and water availability regimes depends on nuanced consideration of the integrative biology of individual taxa on a case-by-case basis.

One important observation discussed recently is that the climatic conditions in regions in which morphologically analogous succulent taxa occur are not as comparable as previously

assumed (Alvarado-Cárdenas et al., 2013; Holtum et al., 2016; see also Moncrieff et al., 2015). By definition, the florae of hotspots of succulent diversity are composed of a high proportion of endemics. While there is a long tradition of analysing endemicity in the context of phylogenetic identity, emphasis should now be placed on establishing the relationships between endemicity, form and function. Even where characteristic taxa of different geographical regions appear superficially analogous in morphology, they may diverge in physiological function thanks to subtle dissimilarities in anatomy.

Water limitation is not only a function of macroclimatic variation; the connection between the epiphytic habit and adaptations for conservative water use has long been acknowledged. Epiphytism is characteristic of several major radiations of vascular plants, including polypod ferns, epidendroid orchids, bromeliads, gesneriads, many of which are succulent (Nyffeler and Eggli, 2010). Although not all epiphytes show pronounced succulence, it is notable that very low degrees of succulence are most common in epiphytes that have evolved phytotelmata as external hydraulic capacitors (e.g. the tank bromeliads; Males, 2016). Selection for succulence is maintained even among epiphytes inhabiting montane cloud forests (e.g. Gotsch et al., 2015) and temperate rainforests (e.g. Godoy and Gianoli, 2013), underlining the difficulties of water acquisition in the absence of soil rooting.

Succulent plants are also well represented in alpine environments. Temperate examples including species in genera such as *Sedum* L. and *Sempervivum* L. in the Crassulaceae (Codignola et al., 1990), while tropical examples include giant rosette species in *Espeletia* Mutis ex Bonpl. In Humb. & Bonpl. (Asteraceae) and *Lobelia* L. (Campanulaceae; Carlquist, 1994). Plants growing at high elevations experience numerous intense environmental pressures, often including water limitation, but also extreme temperatures and ultraviolet (UV) exposure. Succulence may be beneficial with respect to the latter two pressures as well as its more obvious role in plant water economy. The high thermal capacity of massively succulent leaves can effectively uncouple them from low atmospheric temperatures at night, helping to protect cold-sensitive critical tissues (i.e. the shoot apical meristem; Nobel, 1988). Morphological adaptation including pubescence can also modulated night-time leaf temperatures (e.g. Keeley and Keeley, 1989). Many succulents inhabiting locations where temperatures drop below 0°C also display structural or biochemical adaptations to avoid freezing injury (e.g. Nobel and De La Barrera, 2003). High temperature tolerance is also

common in alpine succulents (e.g. Larcher et al., 2010). Alongside the epidermal specialisation to improve UV reflectance (Mulroy, 1979) and high investment in antioxidant phenolics (Bachereau et al., 1998) that are often observed in alpine succulents, it is possible that species with peripheral hydrenchyma could benefit from increased UV reflectance by this tissue.

Halophytes are often described as using succulence to cope with physiological drought rather than the physical water shortage faced by drought-avoidance succulents. Succulent halophytes are epitomised by species of the Chenopodioideae and Salicornioideae (Amaranthaceae; Flowers and Colmer, 2015). However, halophytes are very different in their water-use strategies and their relationship with succulence. Ogburn and Edwards (2010) suggested that succulence in halophytes is primarily a by-product of ionic accumulation in enlarged vacuoles and does not provide capacitance. Halophytic succulence is therefore an almost completely distinct phenomenon, and there are very few examples of angiosperm lineages that display both halophytic and drought-avoidance succulence (Ogburn and Edwards, 2010).

The physiology of succulent water use

The physiology of water use in succulent plants varies more widely than is often suggested. While transpiration rates are strongly restricted in xerophytic drought-avoidance succulents, it has long been recognised that they can be relatively high in succulent halophytes (Delf, 1911, 1912). Among drought-avoidance succulents, two contrasting strategies can be identified in terms of the seasonal dynamics of stored water use. These two strategies are closely connected to life-history.

In small annual succulents, including many Aizoaceae, succulent organs represent single-use water stores that can extend the growing season into the portion of the year defined by less favourable climatic conditions, and depletion of the store coincides with seed production and senescence (Ogburn and Edwards, 2015). A very different type of hydraulic behaviour is observed in storage succulents. These plants display a distinctive water-use strategy involving translocation of water from succulent storage tissue to chlorenchyma during seasonal drought (e.g. Nobel, 2006), buffering chlorenchyma water potential, followed by

refilling of hydrenchyma during seasonal precipitation events. The rehydration process can occur quickly (Scalisi et al., 2016), and involves coordinated responses of root and shoot tissues (North et al., 2004; Griffiths, 2013). In some *Agave* species, a network of fine, short-lived 'rain roots' rapidly develops, increasing total root length by 47% in *Agave deserti* Engelm. (Jordan and Nobel, 1984). In the shoot, aquaporins are also important in maximising the conductance of the pathway between the vasculature and the storage tissues (North et al., 2004). Stomatal aperture also increases, generating a stronger transpirational pull that may help to draw water through the plant towards storage tissues as well as towards the stomata (Nobel, 1988).

[FIGURE 34]

A range of anatomical and biochemical factors are likely to influence the capacity for efficient recharge by modifying the overall hydraulic resistance of the root-capacitor pathway and the partitioning of relative resistances between xylary and extra-xylary compartments (Fig. 34). The overall efficiency of the process should be maximised by coordinated evolutionary changes in xylem properties and processes such as the ionic effect (Zwieniecki et al., 2001), but also in the aquaporin profiles and anatomy of both the root and shoot. Interveinal distance is generally positively correlated with succulence due to developmental constraints imposing a limitation on hydraulic connectivity in many succulents (Ogburn and Edwards, 2013). However, in some succulent lineages, there has been convergent evolution of 'three-dimensional' arrangements of vascularisation, in which multiple layers of vascular bundles permeate the mesophyll. This allow hydraulic homogeneity to be preserved in more succulent leaves by maintaining a low path length for water transport between veins and mesophyll cells (Ogburn and Edwards, 2013; Melo-de-Pinna et al., 2016). This innovation has been linked with elevated degrees of succulence and rates of diversification in the Portulacineae and Molluginaceae (Ogburn and Edwards, 2013). Similar phenomena have been described in the vasculature of succulent stems in other taxa (Mauseth, 1993; Carlquist, 2001; Hearn, 2009). Further empirical and modelling work is required to tease apart the anatomical and biochemical traits that determine the efficiency of recharge and of subsequent water retention.

An important aspect of the vascular biology of succulents that warrants further attention in the context of succulence is the organographic disposition of vessel elements in the xylem.

Carlquist (2009, 2012) has pointed out that in monocots, where root and shoot xylem are discontinuous, succulence is generally accompanied by the restriction of vessel elements to the root and the presence of tracheids or at most low-diameter vessels in the shoot. This arrangement is hypothesised to facilitate the rapid uptake of transiently-available water by the root system but low hydraulic conductance and water loss from aerial organs. Preferential loss of conductance in roots and leaves, due either to cavitation or extra-xylary effects, could serve to hydraulically isolate the stem from declining soil water potential, reducing the chances of catastrophic hydraulic dysfunction during extreme drought (Linton and Nobel, 1999, 2001; North et al., 2004). This is an example of hydraulic segmentation. There is currently intense interest in this phenomenon in the plant hydraulics community (Pivaroff et al., 2014; Bouche et al., 2016; Hochberg et al., 2016; Johnson et al., 2016; Savi et al., 2016; Wolfe et al., 2016; Zhu et al., 2016). Many stem succulents are drought-deciduous (e.g. *Adenium* spp.), but how the prevalence of this phenomenon is modulated by the differential distribution of succulence between plant organs remains unclear. It might be expected to be influenced by the construction costs of hydrenchyma and by shoot architecture.

Within angiosperm leaves, extra-xylary resistance is often equal to or in excess of xylem resistance (Cochard et al., 2004), depending on environmental conditions (Ocheltree et al., 2013). This is probably especially true ~~of~~ for succulents, where the extra-xylary hydraulic pathway is long and tortuous. New models have recently been developed to investigate the relationships between extra-xylary leaf anatomy and hydraulic conductance (Buckley et al., 2015, 2017), which should applied to investigate functional differentiation between contrasting succulent anatomies. Furthermore, there is growing evidence that in many plant taxa, the hydraulic conductance of the extra-xylary compartment may be more sensitive to declining water potential than that of the xylem (Scoffoni et al., 2014; Martorell et al., 2015; Trifilò et al., 2016). How these phenomena play out in succulents is not yet known. In many cases, stomatal behaviour is probably sufficiently conservative to minimise the chances of any significant loss of xylem or extra-xylary hydraulic conductance. However, if turgor loss of mesophyll cells is a potential component of extra-xylary hydraulic vulnerability, we might expect this to be particularly important in succulent species. This is because succulents probably lose turgor at relatively modest leaf water potentials due to low osmolarity, as

483 discussed by Martin et al. (2004) in the context of vascular epiphytes. Aquaporins and
484 plasmodesmata are likely to be very important in the hydraulic conductance of succulent
485 organs because of the high degree of cell-cell connectivity associated with succulent
486 anatomy (Steudle et al., 1980; Murphy and Smith, 1998; Buckley et al., 2015). Since
487 aquaporins are the subject of dynamic regulation, they too could play a key role in variable
488 extra-xylary hydraulic conductance, as has been shown in some non-succulent species (e.g.
489 Vitali et al., 2016).

490 The extent to which the hydraulic capacitance of succulent tissues can be dynamically
491 connected to the transpiration stream to buffer transpiration in the face of variable
492 evaporative demand is not clear (Blackman and Brodribb, 2011). Anatomical factors are
493 important determinants of the connectivity between different pools of leaf water, as
494 indicated by rehydration kinetics experiments (Zwieniecki et al., 2007). The physiological
495 processes involved in the remobilisation of stored water in storage succulents warrant
496 further attention, which may require innovation in real-time imaging methodologies.

497 At the distal end of the endogenous transpiration stream, the sensitivity of stomata of
498 succulent plants to environmental stimuli is still poorly documented. It will be interesting to
499 determine whether evidence can be found for modulation of stomatal sensitivity by other
500 tissue-specific hydraulic conductances and capacitances (Ocheltree et al., 2014), or by
501 stomatal density, size or structural diversity (Franks and Farquhar, 2007; Raven, 2014;
502 McAusland et al., 2016).

503 Recognition of interspecific differentiation in water-use strategies has given rise to the
504 elaboration of hydrological niche theory, which is now well-supported for terrestrial plants
505 (Araya et al., 2011; Silvertown et al., 2015). Succulent plants are sometimes caricatured as
506 somewhat monolithic in terms of their water-use strategies, but this is far from accurate.
507 Some terrestrial succulents, including Aizoaceae from coastal southern African deserts are
508 dependent on occult precipitation rather than rainfall (Matimati et al., 2013). Many
509 succulent epiphytes use specialised structures to harvest atmospheric moisture (Reyes-
510 García et al., 2008; Zotz and Winkler, 2013). The morphological and physiological variety
511 among co-occurring terrestrial succulents has been shown to support hydrological
512 partitioning (February et al., 2013), and recent modelling efforts have demonstrated how

succulent drought-avoidance strategies can coexist with drought-tolerance strategies under water-limited conditions (Manzoni et al., 2014).

Evolutionary developmental biology of succulence

The mechanistic basis of the evolution of succulence remains a puzzle. Relatively little work has been undertaken to explore genetic and ontogenetic mechanisms associated with succulence, or how these differ in storage and all-cell succulence. Hypothetical sequences of stages of structural and physiological specialisation during the evolution of storage and all-cell succulence are outlined in Fig. 43. These evolutionary pathways remain largely unexplored, and invite many intriguing questions. For example, are the evolutionary paths to these two types of succulence rigidly parallel from an early stage, or is it possible to 'jump' from one to the other? Well-resolved phylogenies of key clades are needed to explore these issues, but also better characterisation of the structural detail and selective advantages of different anatomies. At present, we can begin to speculate about how some of the evolutionary changes might have come about.

[FIGURE 43]

Although apoplastic water and mucilage can make an important quantitative contribution to succulence (Nobel et al., 1992; Ogburn and Edwards, 2009), the largest reservoir of water and that which is under the tightest physiological control resides within living cells (Ogburn and Edwards, 2010). Cell size is therefore an important determinant of succulence. Many factors influence cell size (Marshall et al., 2012), among which is nuclear genome size (Beaulieu et al., 2008). Available data are currently too limited to test for a link between genome size and succulence in a phylogenetically-structured manner. As an alternative to genome size, ploidy level can vary. Polyploidy has been documented in many succulent lineages, but there has been no attempt at systematic review to identify correlations with succulence. Ploidy can also vary within the body of the plant, a phenomenon known as endopolyploidy or endoreduplication. De Rocher (1990) identified a role for endopolyploidy in the development of succulence in *Mesembryanthemum crystallinum* L., and similar observations have been made in other succulent species (Braun and Winkelmann, 2016). Mishiba and Mii (2000) found higher levels of endopolyploidy in the large hydrenchyma cells

of *Portulaca grandiflora* Hook. than in smaller chlorenchyma and bundle sheath cells. The genetic and developmental determinants of endopolyploidy are not well characterised, but some key regulators have been identified in Arabidopsis, including the *STRUWWELPETER* (*SWP*) gene (Autran et al., 2002).

Cell size is also affected by the macromolecular content of the cytoplasm, which is controlled by translational regulators such as *TARGET OF RAPAMYCIN* (*TOR*) and *ErbB-3 BINDING PROTEIN1*. Overexpression of these factors in Arabidopsis leads to increases in cytoplasmic protein content and cell size (Horváth et al., 2006; Deprost et al., 2007). The vacuolar contribution to cell volume is also important, representing over 90% of the cell volume in succulents (Gibson, 1982; Von Willert et al., 1992). Increased vacuolar ATPase activity is associated with larger cells in Arabidopsis (Ferjani et al., 2013), but otherwise little is known regarding factors controlling vacuole size.

For a protoplast to increase in volume, the cell wall must also expand. Overexpression of *EXPANSIN10* in Arabidopsis causes an increase in cell size (Cho and Cosgrove, 2000), and Han et al. (2013) have shown that expression of a poplar xyloglucan endotransglucosylase/hydrolase (XTH) transgene in tobacco plants could lead to increased leaf-succulence. Cell wall elasticity is also closely related to capacitance, since it is through changes in cell volume that water is brought in and out of symplastic storage. Cell-cell hydraulic continuity must also be maintained, and the means of achieving this with the greatest potential for dynamic control is to increase the abundance and activity of plasma membrane aquaporins. Qi et al. (2009) demonstrated the importance of an increase in aquaporin activity in the induction of succulence in *Suaeda maritima* (L.) Dumort, while Vitali et al. (2016) have shown that aquaporins can be involved in the determination of hydraulic capacitance in grapevine.

A final consideration in relation to cell size is the phenomenon of compensation. If a mutation causes a decline in cell number, cell size tends to increase in proportion (Hisanaga et al., 2015). The underlying mechanisms of compensation are as yet unknown, as is how the process relates to the evolution of succulence. If maximal succulence is achieved through increases in both cell number and cell size, does this require a loss of function in the machinery of compensation? So far no studies have addressed these issues.

An exciting opportunity in succulent evolutionary development lies in naturally-occurring inducible succulence. This phenomenon is known in a range of taxa, notably certain halophytes on exposure to high concentrations of NaCl (Jennings, 1976; Tiku, 1976; Sui et al., 2010). Physiological drought of this kind has been shown to lead to leaf-succulence through endoreduplication in *Lobularia maritima* (Brassicaceae; Capesius and Loeben, 1983). Succulence is also sometimes induced in response to nutrient deficiencies (Baker et al., 1956; Sharma and Ramchandra, 1989; Sharma et al., 1995), while photoperiod regulates the expression of succulence in some Crassulaceae species (Von Denffer, 1941). Transcriptomic and proteomic comparisons of pre- and post-induction tissues from relevant species could prove extremely illuminating. Indeed, increasing numbers of published genomes and transcriptomes of succulent plants could provide an opportunity for comparative analyses across taxa (Gross et al., 2013; Cai et al., 2015; Ming et al., 2015; Hartwell et al., 2016). Intraspecific variation and phenotypic plasticity in succulence is still little-studied, but, due to the complex cost-benefit scenario inherent in the integration of succulence in leaf structure and function, is probably prevalent and ecologically significant. Chiang et al. (2013) recently showed that in the epiphytic fern *Pyrrosia lanceolata* (L.) Farw., investment in hydrenchyma was strongly influenced by local climatic conditions.

The complex relationship between succulence and CAM

In any discussion of succulence, there is a photosynthetic elephant in the room: Crassulacean acid metabolism (CAM). CAM involves nocturnal stomatal opening and initial fixation of CO_2 by phospho-*enol*-pyruvate carboxylase (PEPC), generating four-carbon organic acids which accumulate in mesophyll cell vacuoles through the course of the night (Osmond, 1978). After dawn, PEPC activity ceases, stomata close, and the stored organic acids are remobilised and decarboxylated to provide extremely high levels of CO_2 for RuBisCO-mediated refixation during the light period. Nocturnal stomatal opening enhances water-use efficiency (WUE) since the leaf-air vapour pressure deficit is generally lower at night, and CAM is therefore classically associated with the same environmental pressures as drought-avoidance storage succulence (Osmond, 1978; Lüttge, 2004). Indeed, because of the requirement for large, highly vacuolate mesophyll cells for organic acid storage in CAM, some degree of succulence is required for CAM to be efficient (Zambrano et al., 2014). The

efficiency of CAM is also improved in densely-packed, thick tissues, partly due to reductions in leakiness between decarboxylation of organic acids and refixation by RuBisCO (Maxwell et al., 1997; Nelson et al., 2005; Nelson and Sage, 2008). Heyduk et al. (2016a) recently used a case of C_3-CAM hybridisation in *Yucca* (Asparagaceae) to provide microevolutionary insights into the coupling of succulence and CAM. Most origins of succulence have accompanied transitions from C_3 to CAM photosynthesis (Ogburn and Edwards, 2010), although it is generally unclear which trait has evolved first, partly because of a paucity of accurate phylogenetic information (Hancock and Edwards, 2014). However, Heyduk et al. (2016b) have recently demonstrated that succulent anatomy predates CAM in the Agavoideae (Asparagaceae), an important radiation of monocot CAM-succulents. Key to further progress in understanding the coordinated evolution of succulence and CAM is the recognition that CAM is a complex syndrome with a continuous scale of functionality rather than a simple binary trait (Silvera et al., 2010; Winter et al., 2015).

Many questions surrounding the wider physiological significance of CAM biochemical rhythms in succulents still need to be comprehensively answered. For instance, it remains unclear how the accumulation of osmotically-active compounds during CAM influences internal movements of water in succulent tissues, or whether they might enhance foliar water uptake (Smith and Lüttge, 1985). Similarly, the complex interactions between acidity levels and other aspects of leaf function in CAM-succulents are still imperfectly understood, despite recent advances (Krause et al., 2016). More fundamentally, gaps in our knowledge of the phylogenetic and geographical distribution of succulence and CAM still hamper efforts to understand their relation to climatic factors (Holtum et al., 2016).

While CAM is the dominant photosynthetic syndrome among succulents, photosynthetic innovation among succulent plants extends to other pathways. Not only is classical C_4 photosynthesis with spatial separation of biochemistry between bundle sheath and mesophyll cells common in succulent halophytes (Sage et al., 2011), but single-cell C_4 systems operate in some Amaranthaceae (e.g. *Bienertia*; Jurić et al., 2016), and unique C_4-CAM species occur in *Portulaca* (Portulaceae; Christin et al., 2014; D'Andrea et al., 2014).

Solving the secrets of succulence

Our understanding of succulence in plants is far from complete. Major questions relating to physiological function, development and evolution remain to be answered. For centuries, succulents have been regarded as curiosities. Eggli and Nyffeler (2009) refer to them as a *Sonderfall*- a special case- because of their unique biology. Historically, relatively few succulents have enjoyed commercial or agricultural significance. These include the pineapple (*Ananas comosus* (L.) Merr.), vanilla orchid (*Aloë vera* (L.) Burm.f.), and *Agave* spp. used for the production of tequila, mescal and sisal; other succulents are important ornamentals (e.g. orchids, *Kalanchoë* spp., succulent geophytes). However, it would be timely now to dispense with connotations of oddness and irrelevance, because there are increasingly many practical reasons to be interested in succulent plants.

There is growing interest in the use of succulent CAM plants (e.g. *Agave*, *Opuntia*) for bioenergy production (Borland et al., 2009; Davis et al., 2011; Holtum et al., 2011; Owen and Griffiths, 2014; Yang et al., 2014; Owen et al., 2016a,b). However, recent studies using new technologies have demonstrated that we do not yet have a clear understanding of the ecophysiological resilience of these plants. Eddy covariance measurements made on a field of *Agave tequilana* F.A.C. Weber plants showed that gas exchange was unaffected even when soil water potential dropped below the threshold identified by previous studies on individual plants (Nobel, 1988; Owen et al., 2016a). Productivity models based on unrepresentative published parameter estimates could therefore generate misleading results, and further work is needed to explore the complexities of the ecophysiological tolerances of bioenergy candidates.

Research programmes are also underway to engineer CAM into C_3 plants for bioenergy and food production (Borland et al., 2015). There are many hurdles to clear on the path to successful engineering of CAM (Borland et al., 2014), including the imposition of succulence to provide sufficient vacuolar storage for malic acid produced during CAM. It is therefore essential to develop systems of reliably inducing functional succulent anatomy, including both increased cell volume, organ volume and cell connectivity.

There are also gains to be made from enhanced knowledge of succulent physiology in ecological applications. Succulents make a major contribution to the biomass and diversity of regions such as the Succulent Karoo, but a disproportionately high number of succulent species are already considered endangered (e.g. Goettsch et al., 2015). Better

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understanding of the physiological ecology of succulent plants is critical to predicting how the vegetation of fragile ecosystems will respond to climate change (Midgley and Thuiller, 2007; Hoffman et al., 2009; Shiponeni et al., 2011; Munson et al., 2012; Schmiedel et al., 2012). While succulent plants show variation in the breadth of their environmental tolerance (Midgley and Thuiller, 2007; Schmiedel et al., 2012), the fitness of any given succulent phenotype is generally highly dependent on bioclimatic context. Research into how the fitness landscapes of different groups of succulent plants are likely to be remodelled by ongoing environmental change should be prioritised. For instance, a combination of empirical and modelling work could be undertaken to explore the sensitivity of long-lived storage succulents to alterations in precipitation regime, taking into account both the direct impacts on water storage and the implications for other plant economic traits including photosynthetic potential.

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Furthermore, improved knowledge of succulent biology may help us to better understand the basis of the economically costly invasiveness of succulents such as *Carpobrotus* N.E.Br., *Lampranthus* N.E.Br., and *Opuntia* spp. (Campoy et al., 2016; Fenollosa et al., 2016).

Box 1. Outstanding Challenges and Opportunities

1. Reconstruction of evolution of succulence by resolution of phylogenetic relationships within major succulent radiations and non-succulent relatives

   Improved computing power for large and complex analyses

   Accessible and easy-to-use packages for analysis of trait evolution and species diversification rates (e.g. 'phytools' for R, Revell, 2012; 'diversitree' for R, FitzJohn, 2012)

2. Identification of fixed and dynamic determinants of pathways of water movement within succulent leaves

   Three-dimensional anatomical microstructure visualisation and functional modelling (Brodersen and Roddy, 2016)

   Experimental silencing of aquaporins using miRNAs and amiRNAs (Sade et al., 2014, 2015)

3. Quantification of variation in functional traits relevant to ecophysiological differentiation across wider range of understudied succulent lineages

   New rapid screening techniques and indices for in situ *characterisation of ecophysiological traits* (e.g. Bartlett et al., 2012; Ogburn and Edwards, 2012; De Kauwe et al., 2016)

4. Identification of molecular factors involved in the induction of succulence in facultative succulents

   Comparative transcriptomic analysis (cf. CAM induction, Brilhaus et al., 2016)

5. Engineering of succulence into non-succulent plants

   Genome editing techniques (Belhaj et al., 2015)

Concluding remarks and future perspectives

Important progress towards understanding the evolutionary physiology of succulence has been made in recent years. We now have a clearer picture of the taxonomic distribution of succulence, the evolutionary trajectory is has taken in certain lineages, and the selective advantages it confers in particular environments. Functional divergence between different

686 succulent anatomies has attracted attention, and this has begun to shed light on links
687 between plant structure and climate relations. A comprehensive portrait of the integrative
688 biology of this large and diverse functional group is a long-term goal that will improve our
689 understanding of plant evolution and support successful exploitation of succulence in
690 applied contexts, and there are many areas to which researchers from different
691 backgrounds can make important contributions (see Box 1, Outstanding Challenges and
692 Opportunities). More robust phylogenies of major succulent lineages and their sister taxa
693 are required to reconstruct the evolutionary origins of succulence in finer detail. New
694 empirical work on structure-function relationships is needed, including studies of the
695 interaction between anatomy and aquaporins in controlling tissue water dynamics. This will
696 help to build better models of succulent water use and make predictions of the responses of
697 succulents to environmental fluctuation in natural and agricultural contexts. The natural
698 diversity of succulents should be exploited through molecular screening methodologies to
699 identify key regulatory factors involved in the induction and development of succulence as a
700 means to facilitating efficient engineering of succulence and CAM.

701

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Figure Legends

Figure 1. Examples of leaf-succulent anatomy, in transverse sectional view. A) *Kalanchoë daigremontiana* Raym.-Hamet & H. Perrier (Crassulaceae), an all-cell succulent; B) *Haworthia cooperi* Baker (Asphodelaceae), a storage-succulent with central hydrenchyma; C) *Tillandsia fasciculata* Sw. (Bromeliaceae), a mixed system with all-cell succulent chlorenchyma and adaxial hydrenchyma. C = chlorenchyma; H = hydrenchyma. Scale bars = 200 µm.

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Figure 21. Global distribution of succulent biodiversity hotspots with names of key taxa (distributions based on Ogburn and Edwards, 2010).

~~Figure 2. Diversity in the spatial arrangement of chlorenchyma and hydrenchyma in leaf storage-succulents, in transverse sectional view. See text for named examples corresponding to each arrangement.~~

Figure 3. Variation in structural and biochemical factors can impact on overall hydraulic resistance to recharge in storage succulents. It can also modify hydraulic design by altering the relative resistances of transpiration stream compartments, including the xylem hydraulic resistance (R_X) and extra-xylary hydraulic resistances of the root ($R_{OX,ROOT}$) and leaf ($R_{OX,LEAF}$).

Figure 4. Hypothetical stages in structural and physiological specialisation during the evolution of the two major types of succulence. The possibility for transitions to occur between either pathway is highlighted. This is an area ripe for investigation by evolutionary developmental biologists.